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Research Article

Phytochemical characterization of *Angelica glauca* root using spectroscopic techniques, GC-MS, and elemental analysis

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Background: Ayurveda mentions numerous plants, among which *Angelica glauca* is renowned for its medicinal and aromatic properties. It is native to the Himalayan region and belongs to the Apiaceae family. The plant has been traditionally used as a spice by the indigenous people of the Himalayas and is commonly known as Chora. The plant may have medicinal value, but scientific evidence for this is lacking. So, the aim of this study is to identify the various phytochemicals present in this plant using different analytical techniques and also evaluate the antioxidant potential using *in vitro* and *in silico* approach.

Methods: For phytochemical identification different analytical techniques were used such as ultraviolet spectroscopy, FTIR and GC-MS. This showed the presence of various phytochemicals belonging to the phenolic, flavonoid, terpenoid, and phthalide classes, with most of the phytochemicals belonging to the terpenoid and phthalide classes. Further, energy dispersive X-ray and scanning electron microscopy were employed for both quantitative and qualitative elemental analysis.

Results: Phenolic and flavonoid concentrations were revealed that their levels were highest in the ethanolic extract with 101.33 ± 5.36 mg GAE/g DW for phenolic content and 54.53 ± 2.23 mg AAE/g DW for flavonoid content. The hydroethanolic extract was found to possess good antioxidant potential, as measured by its total antioxidant capacity, which was determined to be 11.14 ± 0.12 mg AAE/g DW. Furthermore, an IC_{50} value of 208.49 µg/mL was determined in DPPH assays. *In silico* analysis also confirmed the antioxidant potential of the plant, highlighting STAT3, HSP90AA1, and EGFR as the potential genes against oxidative stress.

Conclusion: The primary focus of this study is to identify various phytochemicals present in its roots using a range of analytical techniques, thereby contributing to its phytochemical profiling which will facilitate future research, drug development, and the standardization of herbal formulations derived from this plant.

Keywords: *Chora, essential oil, secondary metabolites, antioxidant activity, In-silico analysis*

Introduction

According to the "World Health Organisation" assessment, approximately 80% of the people living in developing nations depend on traditional medicines for their medical needs. Ayurveda, Siddha, and Unani are among the indigenous medicinal systems in India that depend significantly on ancient herbal treatments and have a long history of use. Ayurveda is the cornerstone of India's traditional medical system and is often referred to as "the science of life". Although the exact antecedents of Ayurveda are unknown but its distinct concepts appear to have developed in India around 2500 and 500 BC. As the oldest recorded medical system, it can be considered to be more beneficial than modern treatments in certain circumstances. *A. glauca* is an important medicinal plant and is used in Ayurvedic medicines for the treatment of many diseases (Table 1). In Ayurvedic literature, this plant is known by several synonyms, including Granthikeshi, Keshini, Nishachari, Kshemika, Dhanya, Dhanvanti, Ganahasa, Kopanaka, Dushpatra, Shankhanika, Phalachoraka, Shukla, Ardrakanda, Taskarah, Kshemaka, Chanda, Ripu, and Kitava (Pillai et al., 2020). This plant generally grows at an elevation between 2,000 and 4,000 m. It is mainly found in Himachal Pradesh, Uttarakhand, and Jammu & Kashmir (Butola et al., 2010; Pillai et al., 2020; Purohit et al., 2015). The plant is commonly known as Chora, Chorak, and Choraka across the Himalayan region (Kumar et al., 2022). *A. glauca* contains various bioactive phytochemicals responsible for its pharmacological properties and aims to treat ailments like rheumatism, fever, asthma, bronchitis, constipation and reproductive issues (Bisht et al., 2006; Butola & Vashistha, 2013; Butola et al., 2016; Thakur et al., 2025). Its rich essential oil content makes it highly sought after in the pharmaceutical, cosmetic, and fragrance industries. Hence useful in aromatherapy and other modern medicinal applications (Rawat et al., 2018). Traditionally, this plant has been utilized as fodder to enhance lactation in livestock, as a culinary spice, for various ethnomedicinal applications, and as a natural snake and insect repellent (Bisht et al., 2006; Kumar et al., 2022). It has been also mentioned extensively in Ayurvedic literature, with both sources emphasizing its therapeutic potential. Although its use in traditional and Ayurvedic medicine is well-established, there is still not much research on its phytochemical profiling and antioxidant potential. So, its antioxidant potential must thus be investigated in order to open the door for its efficient and scientifically supported application in the management of a number of diseases. Thus, the purpose of this study was to investigate *A. glauca* potential as a natural antioxidant.

Table 1. Different ancient Ayurvedic formulations prepared from *A. glauca*

Classical Source	Chapter/ Section Name	Classification/ Formulation	Traditional description / Uses	References
Kautilya's Arthashastra	Chapter 15 of Arthashastra (The Superintendent of Storehouse and)	Tikta Varga	Used as a spice	(Pillai et al., 2020)
Charaka Samhita	Sanjnasthapana Mahakashaya	Sanjnasthapana Mahakashaya	Sheeta nashana, Kushta, Shirashoola	(Acharya, 1972)
	Kumaragara	Fumigation	Fumigation of beds, garments and bedsheet with ghee	(Pillai et al., 2020)
	Unmada Chikitsa or Vata Sleshmatmaka Apasmara Chikitsa	Maha Paishachika Ghrta	Used in Unmada	(Acharya, 1972)
	Apasmara Chikitsa	Dhoopana dravya	Mentioned for Utsadana	(Sharma & Guruprasad, 1979)
	Hikka Shwasa Chikitsa	Shatyadi Churna	Treatment of shwasa or hikka	(Vagbhata, 2016)
	Madana kalpa	Utkarika	Used in preparation of Utkarika	(Vagbhata, 2016)
Ashtanga Hridaya and Ashtanga Sangraha	Eladi gana	Eladi gana	Arunadatta and Hemadri have mentioned it as Granthiparna	(Vagbhata, 2016)
Kasyapa Samhita	Bhutavidya	Dhoopana	Used for Moha	(Jivakiya, 2005)
Dhanvantri Nighantu	Chandanadi varga	Tikta	Mentioned as Krimihara, Vatahara, Kushta kanduhara, Visharaktantakaraka, Vranahara	(Pillai et al., 2020)
Raja Nighantu	Chandanadi varga	Tikta	Mentioned as Nasa Mukha Rogahara,Vata kaphahara,	(Pillai et al., 2020)

			Krmihara, Ajirnahara and Rujahara	
Madanapala Nighantu	Karpooradi varga	Madhura	Mentioned as Vata kaphahara, Kusthahara and Asrajit	(Pillai et al., 2020)
Shaligrama Nighantu	Karpuradi varga	Madhura, Tikta	Mentioned as Kaphahara, Vata vikarahara, Kandu kusthahara, swedahara, Tvak dosahara, Daurgandhyahara, Vranahara, Hrdya, Mukha nasa rujahara, Alaksmi Nasanam, Krmihara, Rakta dosahara	(Pillai et al., 2020)

Materials and Methods

Collection and preparation of extracts

The dried root was collected from a local shop in Shahpur market, District Kangra, Himachal Pradesh, where it is commonly known as Chora roots. The dried roots were then ground into a fine powder and subjected to Soxhlet extraction using three different solvents: distilled water (aqueous), ethanol, and a hydroethanolic mixture (50:50, v/v). For each extraction, 10 grams of the powdered root sample were loaded into a thimble and extracted with 250 mL of the respective solvent until the solvent became colorless, indicating complete extraction. Then the resulting extracts were filtered, and the solvents were evaporated using a rotary evaporator. The obtained extracts were collected and stored for further analysis. For essential oil extraction Clevenger apparatus was used. 700 mL of distilled water was added to a distillation flask along with 100 g of the dried root material. The essential oil was extracted by allowing the apparatus to run for 3-4 hours. After that, the collected essential oil was stored at a cold temperature for further analysis.

Characterization techniques

UV analysis

For UV (Ultraviolet) spectral analysis, the extract was initially prepared using the same solvent in which it was extracted, i.e., distilled water, ethanol, and hydroethanol. The prepared extract was then centrifuged (for 10 min at 3000 rpm) to remove any insoluble debris, and the clear supernatant was collected. A tenfold dilution of the supernatant was made using its original solvent as diluent. The diluted sample was transferred into quartz cuvettes for spectrophotometric analysis. UV absorption spectra were recorded using a Thermo Scientific spectrophotometer across the wavelength range of 200 to 800 nm. Then the observed spectra were matched or checked with the available data for identification of different phytochemical classes (Mabasa et al., 2021).

FTIR analysis

FTIR analysis was used for identification of different functional group using the Perkin Elmer FTIR instrument. The infrared spectra were recorded using the standard scanning protocol (Singh et al., 2024). The resulting spectra were compared with established reference data to identify the functional groups in the *A. glauca* roots (Nandiyanto et al., 2019).

SEM and EDX

The surface morphology of *A. glauca* root dried powder was examined using a SEM (Scanning Electron Microscopy) (Carl Zeiss SUPRA 55), and to confirm the elemental composition of the root sample, EDX (Energy Dispersive X-ray analysis) was performed using an Oxford Instruments EDS detector coupled with the SEM system (Singh et al., 2024).

Phytochemical analysis of volatile components using Gas Chromatography-Mass Spectrometry

The essential oil extract was analyzed by GC/MS-QP2010 system. A non-polar ZB-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness) was used for separating the metabolites. Helium was used as the carrier gas, at 1.05 mL/min. An initial period of isotherm at 70 °C for 5 min on the GC oven was reserved, followed by a temperature gradient of 4 °C/min until 220 °C with a maintenance time of 5 min to guarantee complete elution of more volatile components. The mass range was 40-800 m/z, and the ionising energy was set to 70 eV. Whereas, the injector's

temperature was kept at 240 °C and interface temperature set as 250 °C. The final sample was made in DCM at a concentration of 10 mg/1.5 mL, and 2 µL was employed in split mode and 10:1 ratio for injection. To determine the Arithmetic Index (AI), a standard mixture of n-alkane chains (C8–C25) was injected into the GC/MS.

In vitro Analysis

Total phenolic content assay

TPC of the plant extracts was determined using the Folin–Ciocalteu colorimetric method with slight modification (Do et al., 2014). A stock solution of 5 mg/mL was prepared, from which 0.1 mL was used for further analysis. 0.1 mL plant extract was diluted with the appropriate solvent to make a final volume of 1 mL. After that, 10% FC reagent (500 µL) was added to the plant extract. After 5 minutes, Na_2CO_3 (400 µL) was added to the test tubes and incubated in the dark at room temperature. After half an hour, the absorbance was observed at 765 nm, and the values were calculated as mean ± SEM. In this, gallic acid as a standard was used with different concentrations, and the experiment was performed three times.

Total flavonoid content assay

TFC of the plant extracts was determined using the aluminum chloride colorimetric method with slight modification (Do et al., 2014). A stock solution of 5 mg/mL was prepared, from which 0.1 mL was used for further analysis. 0.1 mL plant extract was diluted with the appropriate solvent to make a final volume of 1 mL. After that, 10% $AlCl_3$ (0.3 mL) and CH_3COOK (0.3 mL) were added to the test tubes and incubated in the dark at room temperature. After 30-40 minutes, the absorbance was observed at 415 nm, and the values were calculated as mean ± SEM. In this experiment, ascorbic acid was used as a standard at different concentrations, and the experiment was performed three times.

Total antioxidant capacity assay

The TAC of the plant extracts was assessed using the phosphomolybdenum method (Zengin et al., 2015). First, a stock solution of 5 mg/mL of plant extract was made. A mixture of 3 mL of the reagent solution and 0.1 mL of plant extract was incubated at 95°C for 90 minutes in a water bath. After cooling the tubes, the absorbance at 695 nm was measured using UV-Vis spectrophotometer. Standard Ascorbic acid was applied, and a calibration curve was prepared with various concentrations. Total antioxidant contents (as milligrams of ascorbic acid equivalents per gram of dry weight) were used for calculation. The values were expressed as mean ± SEM, and the experiment was conducted in triplicate.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The DPPH assay was used to determine the free radical scavenging activity of different root extracts and essential oil using the methodology described by (Chaudhary et al., 2014). 1 mL of plant extracts at several concentrations was mixed with 1 mL of DPPH solution prepared in methanol. After that, the mixture was left at room temperature for 20 to 30 minutes in the dark. Then at 517 nm absorbance it was measured using a UV–Vis spectrophotometer.

Methanol was employed as a blank, whereas DPPH solution alone, without the sample was used as a control, and ascorbic acid was used as a positive control. Then a graph was plotted between the % of scavenging activity and extract concentration, from which the IC_{50} value was obtained.

The radical scavenging activity was calculated using formula:

$$\% \text{ DPPH radical scavenging activity} = \frac{AC\text{-}AS}{AC} \times 100$$

Where, AC represents by absorbance of the DPPH blank and AS represents the absorbance of the plant extract.

Statistical analysis

The experiments were performed in the triplicates and presented as mean ± SEM. The statistical software OriginPro was used to evaluate variances and interactions at a 0.05 significance level, employing analysis of variance (ANOVA) and post hoc comparisons using Tukey's test.

In-silico analysis

Screening of phytochemicals and target identification

Phytochemicals present in this plant were initially identified through GC–MS analysis. A total of 13 compounds were detected and evaluated for drug-likeness using the SWISS ADME platform (http://www.swissadme.ch/). Compounds meeting the criteria of a bioavailability score ≥ 0.55 and also match the standards of the Lipinski's rules of five were screened for further analysis. Target prediction for the filtered phytochemicals was carried out using the Swiss Target Prediction tool (http://www.swisstargetprediction.ch/). To associate the predicted targets with antioxidant potential, the Gene Cards database (https://www.genecards.org/) was queried using the keyword "oxidative stress" and the results were filtered using a relevance score > 10 to identify the most relevant genes. Finally, Venny 2.1.0 was employed to identify the overlapping targets between the predicted phytochemical targets and antioxidant-related genes, providing valuable insights into their potential antioxidant roles (Oliveros, 2007).

Protein–protein interaction (PPI) network analysis

To explore the interaction dynamics of the common targets, the STRING database (https://cn.string-db.org/) was utilized for constructing a protein–protein interaction (PPI) network with the highest confidence (≥ 0.9). Genes were represented as nodes in the resulting network, while edges indicated both functional and physical interactions. Each node was assigned a degree value, reflecting the number of interactions it holds within the network. The generated network was then imported into Cytoscape software for further topological analysis and visualization. To pinpoint the most influential genes, the CytoHubba plugin was applied, allowing the identification of hub genes based on their degree centrality (Thakur et al., 2023).

Enrichment analysis

Enrichment analyses for GO and KEGG pathways were conducted using DAVID (https://david.ncifcrf.gov/) to assess the biological relevance of common targets between *A. glauca* and oxidative stress. Then, the bioinformatics tool (https://www.bioinformatics.com.cn/en) was utilized to illustrate the findings, providing insights into their potential role as antioxidants.

Molecular docking

Following network analysis, it was observed that STAT3 possesses the highest degree score and interacts with Z-Ligustilide. Therefore, a molecular docking approach was employed to investigate the interactions between STAT3 and the potential target compounds. The protein structure was retrieved from the RCSB PDB (https://www.rcsb.org/) database using PDB ID: 6NJS, while the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database was utilized to obtain the 3D structures of the compounds. AutoDock Tool 1.5.7 was then used to prepare protein and ligand structures for docking. First, water molecules were removed from the protein structure, after which appropriate charges were added using the AutoDock Tool. Subsequently, the prepared ligand was docked into the active sites of the protein using AutoDock Vina (https://vina.scripps.edu/) (Trott and Olson, 2010). After that, Discovery Studio was used for analysis of key molecular interactions.

Results

UV analysis

In order to find phytochemicals, the UV spectral characterisation was used to identify compounds with π-bonds, non-bonding electron pairs, conjugated systems, and aromatic rings. These compounds absorb light in the UV region of the electromagnetic spectrum, which is usually between 200 and 800 nm. The final graphs, however, were plotted between 200 and 400 nm since the spectra beyond that point showed minimum variation with no notable peaks (Table 2). The observed absorption peaks correspond to the presence of different classes of phytochemicals. In the aqueous extract, a series of 10 prominent peaks were observed between 200–280 nm, which typically indicate the presence of phenolic compounds and flavonoids, known to strongly absorb in the UV region due to their conjugated systems. Additionally, three peaks were noted between 300–350 nm, which may be attributed to flavanols, flavones, or other polyphenolic compounds. In the ethanolic extract, 11 peaks were observed in the 200–280 nm range, suggesting the abundance of phenolics and flavonoids, while four peaks were detected in the 300–350 nm region, also indicating the presence of flavonoids and other secondary metabolites. The hydroethanolic extract showed ten peaks of flavonoids and other related

compounds in the 200–280 nm and at 300–350 nm ranges showed six prominent peaks, indicating the presence of these phytochemicals while the essential oil from root revealed 12 peaks in the 200 to 290 nm zone mostly related to terpenoids and aromatic compounds that are naturally present in volatile oil and also some phenolic and flavonoid compounds from 300–350 nm region. Overall, these spectral findings revealed the presence of various phytochemicals.

Table 2. UV spectral data of *A. glauca* root

Absorption maxima (wavelength ranges)	Phytochemical compounds	References
200-250 nm	Terpenoids	(Lin et al., 1998)
200–280 nm	Phenolic compounds and flavonoids	(Alexander et al., 2023)
250–300 nm	Terpenoids	(Lin et al., 1998)
234–676 nm	Flavonoids, alkaloids, phenolic compounds	(Patle et al., 2020)
300–350 nm	Flavonoids, flavonols, flavones, or other polyphenolic compounds	(Deliza et al., 2023)

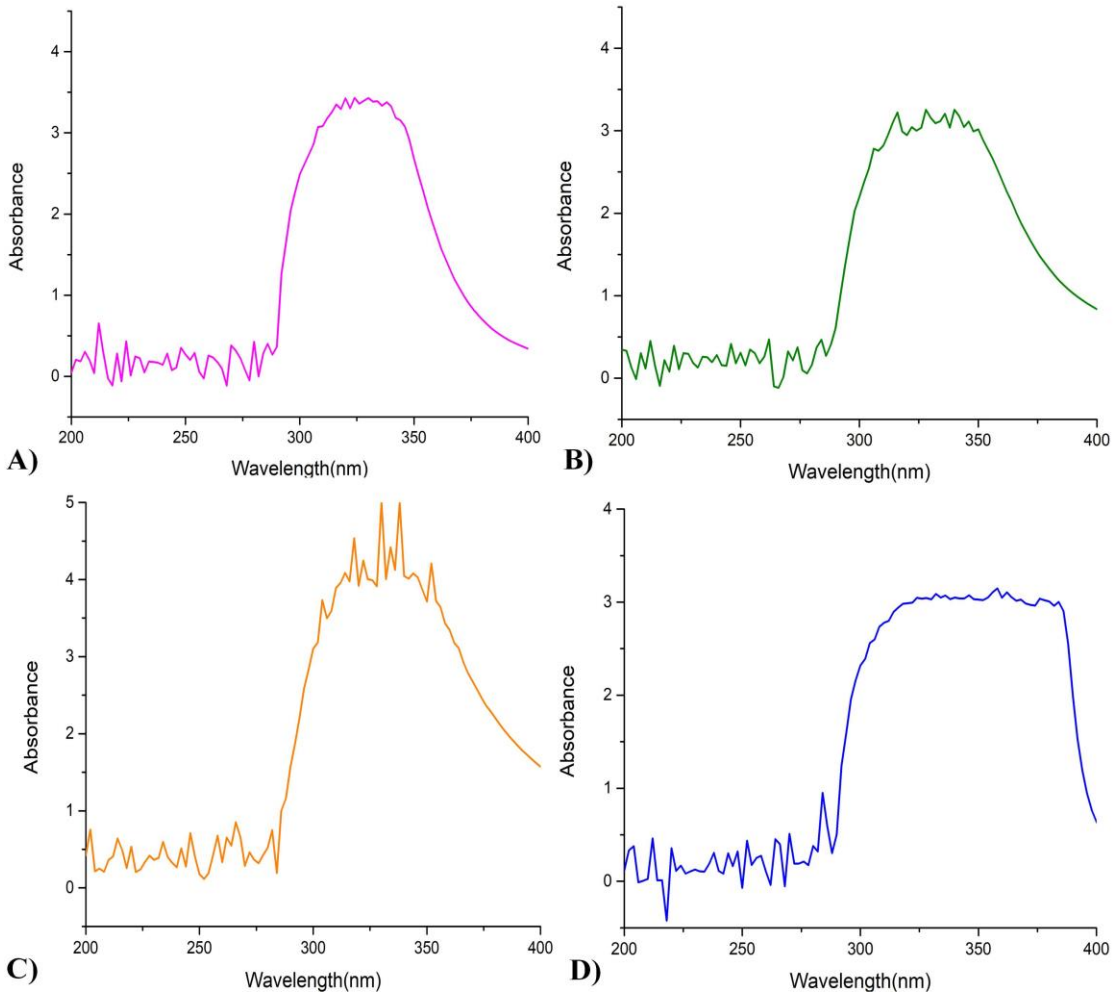

Figure 1. UV spectrum of different root extracts and essential oil (200–400 nm) showing multiple characteristic peaks of phytochemicals; (A) *A. glauca* market root aqueous extract (AGMRA); (B) *A. glauca* market root ethanolic extract (AGMRE); (C) *A. glauca* market root hydroethanolic extract (AGMRH); (D) *A. glauca* market root essential oil (AGMRO)

FTIR analysis

For the identification of the functional groups prevalent in different plant extracts, FTIR spectroscopy was used. The basis of this approach is the absorption of infrared light at particular wavelengths that correlate to functional group vibrational transitions. When the extract was subjected to FTIR analysis, distinct peaks were observed, indicating the presence of various functional groups (Figure 2). The FTIR spectrum of the aqueous and ethanolic extracts revealed

seven major absorption peaks, while the hydroethanolic extract showed thirteen prominent peaks mentioned in Table 3. However, the essential oil sample showed fifteen prominent absorption peaks as described in Table 3.

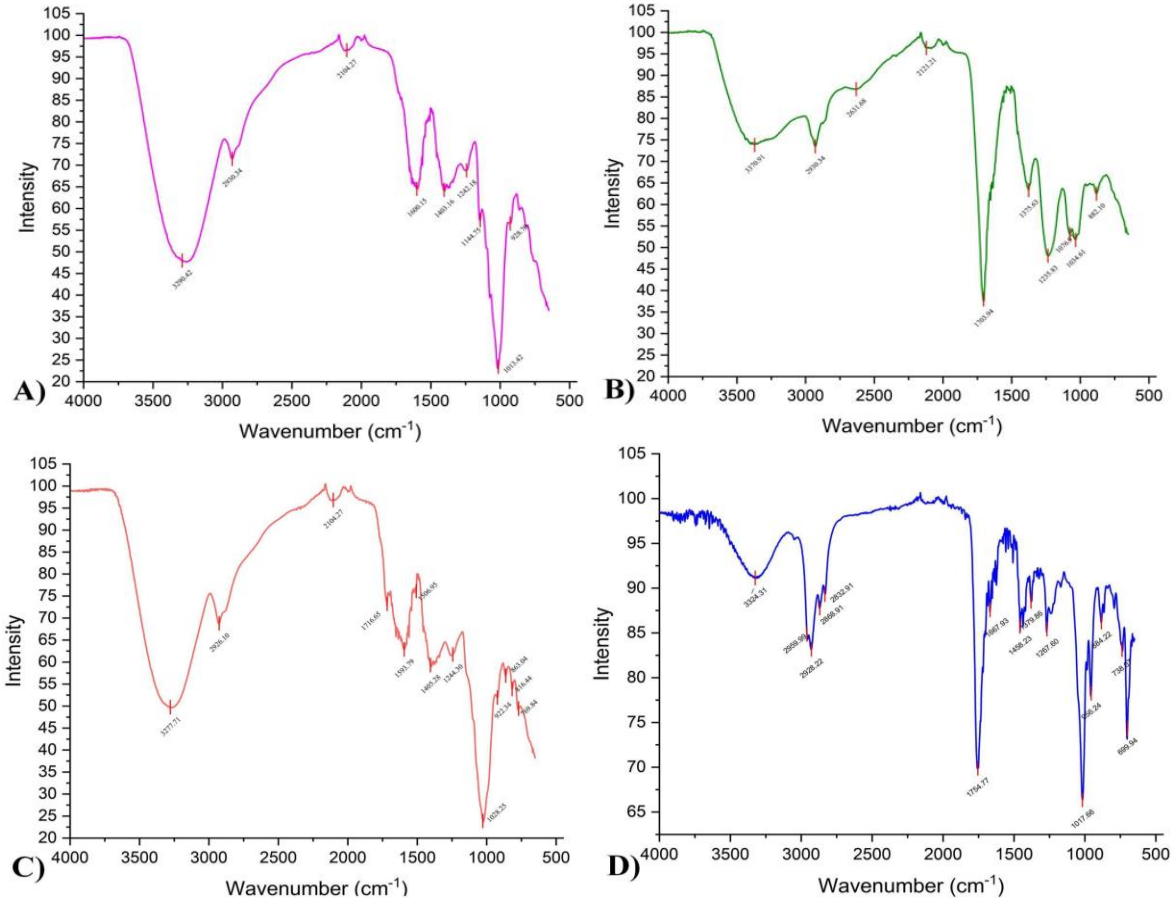

Figure 2. FTIR spectrum analysis of the different root extracts and essential oil of *A. glauca*; (A) *A. glauca* market root aqueous extract (AGMRA); (B) *A. glauca* market root ethanolic extract (AGMRE); (C) *A. glauca* market root hydroethanolic extract (AGMRH); (D) *A. glauca* market root essential oil (AGMRO)

Table 3. FTIR data of *A. glauca* root

Sr. No.	Frequency ranges	Frequency Peak Values				Vibration/bond	Specific functional group	Chemical compound
		AG-MAQ	AG-MET	AGMHE	AG-MEO			
1.	3570-3200	3290	3370	3277	3324	OH stretch	Alcohol and hydroxy compound	Phenols and Flavonoid
2.	1410-1310	1403	1375	1405	1379	OH bend		
3.	1270-1230	1242	1235	1244	1267	aryl-O stretch	Aromatic Ether	
4.	2850–2815	-	-	-	2832	O-CH_3	Methyl ether	Flavonoid and Terpenoid
5.	2970–2950/2880–2860	-	-	-	2868, 2959	C-H Stretch	Methyl	
6.	2935–2915	2930	2930	2926	2928	C-H Stretch	Methylene	
7.	1055-1000	1013	1034	1028	1017	C-H Bend	Methylene (Cyclohexane ring vibrations)	
8.	1300-700	-	-	922	956	C-C vibrations	Saturated aliphatic	

9.	1650-1590	1600	-	1593	-	NH bend	Primary amine	Terpenoids
10.	1760–1740	-	-	-	1754	C=O stretch	Alkyl carbonate	Flavonoid and Terpenoid
11.	1725–1700	-	1703	1716	-	C=O stretch	Carboxylic acid	
12.	1680–1630	-	-	-	1667	C=O stretch	Amide	Terpenoids
13.	1510-1450	-	-	1506	1458	C=C-C stretch	Aromatic ring (aryl)	Flavonoid and Terpenoid
14.	900-670	-	-	863, 816, 769	884, 738,699	C-H out-of-plane bend	Aromatic	

AGMRA: *A. glauca* market root aqueous extract; **AGMRE**: *A. glauca* market root ethanolic extract; **AGMRH**: *A. glauca* market root hydroethanolic extract; **AGMRO**: *A. glauca* market root essential oil.

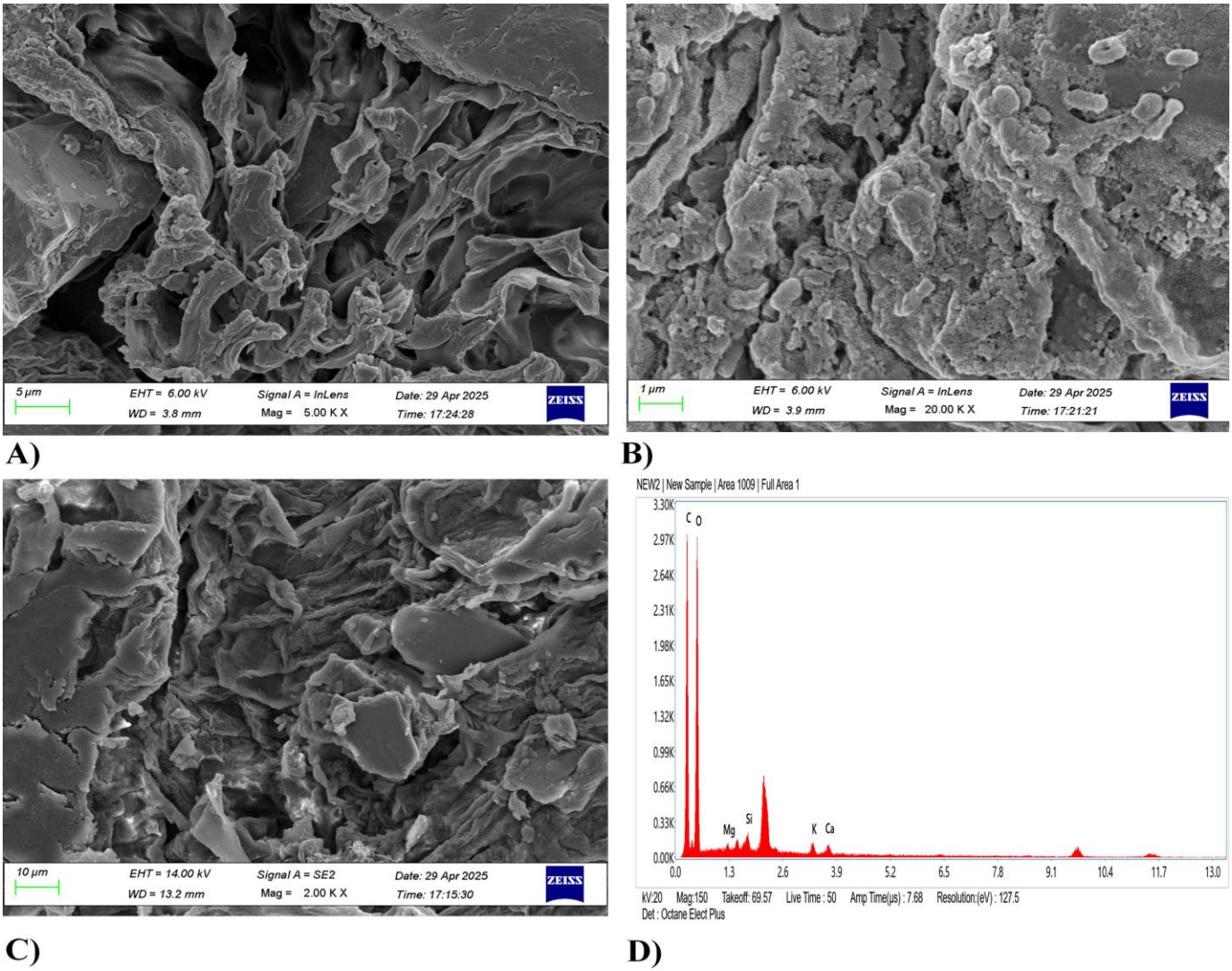

Figure 3. SEM images of area scanned for elemental analysis of root sample (A) Scan-I (5 µm, 6.00 kV, magnification 5.00 K X], (B) Scan-II [1 µm, 6.00 kV, magnification 20.00 K X], (C) Scan-III [10 µm, 14.00 kV, magnification 2.00 K X]; (D) EDX spectra of root sample for elemental analysis

SEM and EDX Analysis

The surface morphology of the root was examined using SEM (Figure 3A-C). EDX Analysis was carried out to determine the elemental composition of the plant root sample. From the SEM and EDX analysis, it was observed that carbon and

oxygen were present in high amounts, compared to Mg, Si, K, and Ca, which were present in very low amounts (Table 4 & Figure 3D).

Table 4. EDX data for elemental composition in *A. glauca* root powder

Sr. No.	Element	Symbol	Weight (%)
1.	Carbon	C	55.71
2.	Oxygen	O	42.42
3.	Magnesium	Mg	0.28
4.	Silicon	Si	0.58
5.	Potassium	K	0.48
6.	Calcium	Ca	0.54

GC-MS analysis

A total of 13 compounds were identified in the dry root essential oil using GC-MS analysis (Table 5) (Supplementary Figure 1). The major constituents were found to be E-Butylidene Phthalide (23.38%), Z-Ligustilide (22.11%), and Z-Butylidene Phthalide (17.81%), among others.

Table 5. Essential oil composition of *A. glauca* root

Peak	R. Time	Area%	Name	RI	Pub Chem id
1	17.912	0.77	1,2-Benzenedicarboxylic acid, monomethyl ester (CAS) Methyl hydrogen phthalate	1311	1017
2	19.061	2.54	1,4-Cyclohexadiene-1,2-dicarboxylic anhydride	1345	138348
4	25.305	1.35	Kessane	1532	11310616
5	26.808	8.94	(-)-Spathulenol	1580	13854255
7	28.758	1.47	T-Cadinol	1645	160799
8	28.966	2.06	Butylphthalide	1652	61361
9	29.084	0.75	Alloaromadendrene	1656	10899740
11	29.585	17.81	Z- Butylidene Phthalide	1672	642376
14	30.943	23.38	E- Butylidene Phthalide	1720	5352899
16	31.318	22.11	Z-Ligustilide	1734	5319022
17	33.029	2.26	E-Ligustilide	1798	5877292
18	36.532	1.09	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate/ Methyl pentadecanoate	1924	23518
19	40.705	2.72	9,12-Octadecadienoic acid, Methyl Ester (CAS) Methyl linoleate	2089	3931

Characterization of total phenolic content

From the Folin-Ciocalteu assay, it was observed that the highest phenolic content was present in the ethanolic extract (AGMRE=101.33 ± 5.36 mg GAE/g DW), followed by the hydroethanolic extract (AGMRH), which shows 60.22 ± 5.48 mg GAE/g DW of phenolic content. While low phenolic content was observed in the aqueous extract (AGMRA) and essential oil (AGMRO), with phenolic concentration of only 27.88 ± 4.01 mg GAE/g DW and 15.11 ± 3.10 mg GAE/g, respectively. Highly significant differences were observed in the total phenolic content among all extracts ($p<=0.001$) (Figure 4A).

Characterization of total flavonoid content

The flavonoid content was quantified using the aluminum chloride assay and expressed as mg of ascorbic acid equivalents per gram of dry weight (mg AAE/g DW). The aqueous extract (AGMRA) exhibited 10.58 ± 2.10 mg AAE/g DW, ethanolic extract (AGMRE) showed 54.53 ± 2.23 mg AAE/g DW, hydroethanolic extract (AGMRH) showed 36.78 ± 3.63 mg AAE/g DW, whereas the essential oil (AGMRO) from roots showed 7.4 ± 3.11 mg AAE/g DW of flavonoid content respectively. Significant differences were observed in total flavonoid content among the extracts, with AGMRE showing significantly higher content than AGMRA, AGMRH, and AGMRO ($p<=0.001$), while AGMRA and AGMRO showed a significant difference ($p<=0.05$) (Figure 4B).

Assessment of total antioxidant capacity

The TAC of the extract was evaluated using the phosphormolybdenum assay and expressed in terms of ascorbic acid equivalents (mg AAE/g DW). The substantial capacity of the different plant extracts to convert molybdenum (VI) to molybdenum (V) indicates the antioxidant potential of the plant. The TAC was observed to be 5.09 ± 0.20 mg AAE/g DW in aqueous extract (AGMRA), 8.71 ± 0.08 mg AAE/g DW in ethanolic extract (AGMRE), 11.14 ± 0.12 mg AAE/g DW in hydroethanolic extract (AGMRH), whereas 7.33 ± 0.19 mg AAE/g DW in essential oil (AGMRO). Significant differences were observed in TAC among the extracts, with AGMRH exhibited significantly higher TAC than AGMRA and AGMRO ($p<=0.001$), and significantly higher TAC than AGMRE ($p<=0.01$) (Figure 4C).

Assessment of radical scavenging activity

The DPPH assay demonstrated significant antioxidant potential of the different plant extracts, revealing a progressive increase in radical scavenging activity in a dose-dependent manner. Significant differences were observed among the extracts at concentrations of 40–180 µg/mL ($p<=0.05$–0.001). The maximum scavenging activity was observed in hydroethanolic extract (AGMRH) with a 208.49 µg/mL IC_{50} value. Whereas, a 227.81 µg/mL IC_{50} value was observed in ethanolic extract (AGMRE), a 273.52 µg/mL IC_{50} value in aqueous extract (AGMRA), and a 317.09 µg/mL IC_{50} value in essential oil (AGMRO) (Figure 4D).

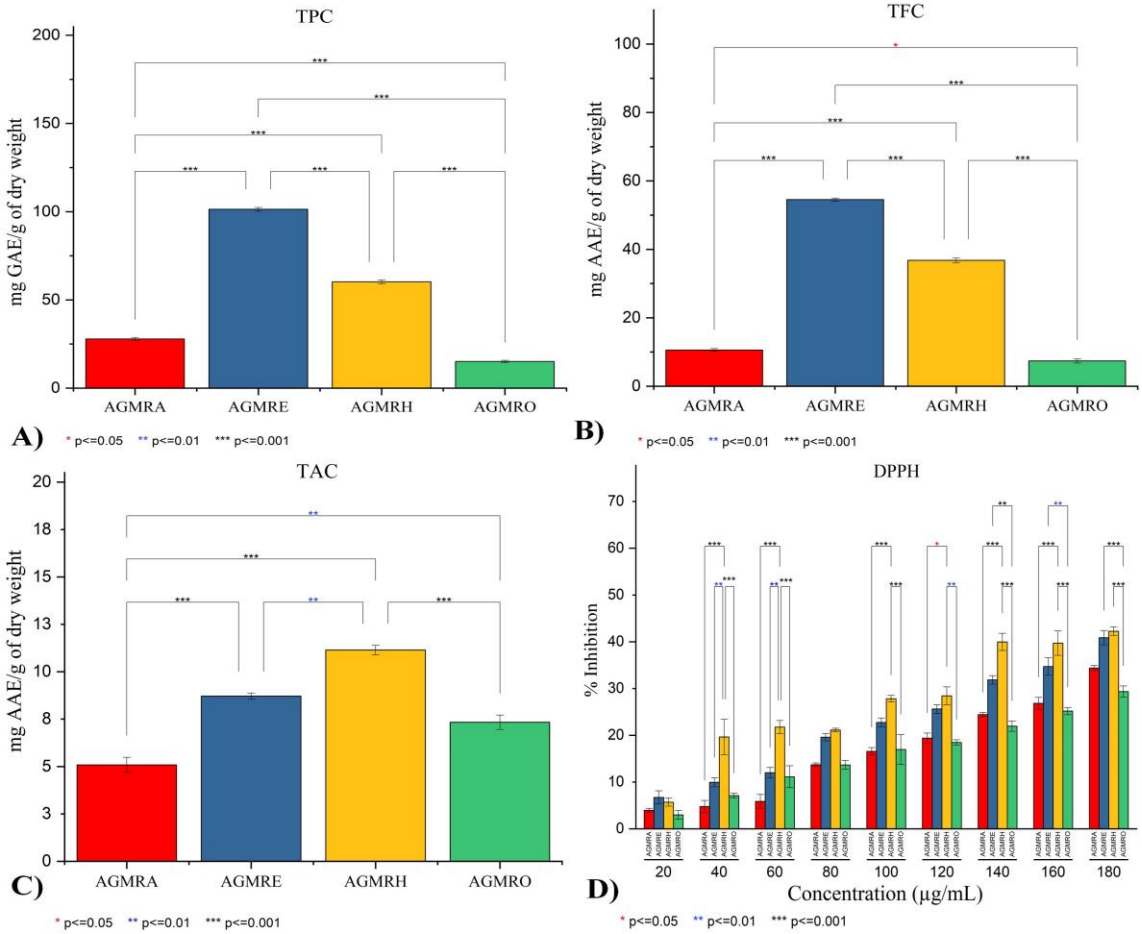

Figure 4. (A) Assessment of total phenolic content present in different root samples (expressed as GAE); (B) total flavonoid content present in different root samples (expressed as AAE); (C) total antioxidant capacity observed in different root samples (expressed as AAE); (D) antioxidant activity of different root samples using the DPPH radical scavenging assay

Target identification & screening and PPI network analysis

Initially, the bioactive compounds were identified through GC-MS analysis. A total of 13 major compounds (Table 5) were selected for further *in silico* evaluation. The target prediction for these compounds was carried out using the http://www.swisstargetprediction.ch/ databases, resulting in the identification of 441 potential protein targets. After

removing duplicate entries, the disease-related target genes were retrieved from the GeneCards database. Subsequently, a common target analysis was performed using Venny 2.1 to determine the common targets between compound-associated and disease-related genes, which yielded 265 overlapping targets (Figure 5A). The 265 common targets were subsequently subjected to PPI network analysis using the STRING database. The resulting STRING interaction network comprised 540 nodes and 3072 edges (Figure 5B), which was visualized and further analyzed using Cytoscape software. Then, the top hub genes representing the most promising molecular targets were identified as STAT3, HSP90AA1, EGFR, MAPK1, PIK3CA, ESR1, PIK3CD, PIK3CB, MAPK3, and PTPN11 using CytoHubba based on the degree centrality score (Figure 5C).

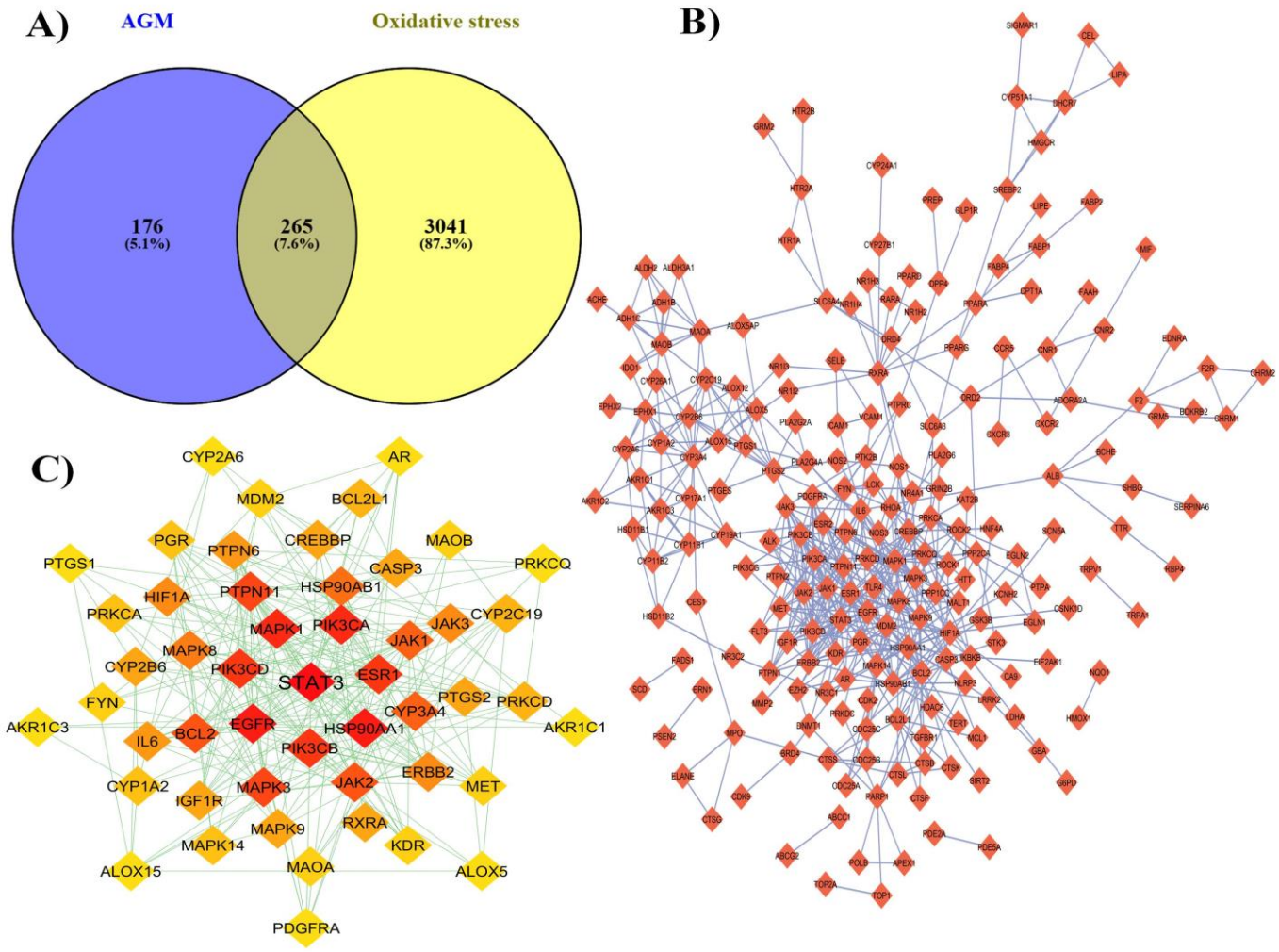

Figure 5. (A) Common targets between *A. glauca* roots and oxidative stress; (B) PPI network of common potential targets with 540 nodes and 3072 edges; (C) PPI network of top 50 hub gene in which dark colour and large size represent more degree score

GO and KEGG pathway analysis

To determine the biological significance of the common targets, we performed functional enrichment analysis. These targets have been linked in 827 pathways connected with biological processes (BP), 101 pathways associated with cellular components (CC), and 269 pathways associated with molecular functions (MF), according to the Gene Ontology (GO) analysis (Supplementary Sheet 1). To further elucidate the therapeutic potential of plant KEGG enrichment pathway analysis was also conducted, revealing a total of 169 pathways. The top pathways involved in gene ontology and KEGG analyses were highlighted in Figure 6.

Molecular docking

A molecular docking study investigated the binding affinity and molecular interactions between the ligand Z-ligustilide and the protein STAT3. The binding energy for the docked compound was calculated to evaluate the strength of the

interactions between STAT3 and the potential ligands, yielding a value of -5.8 kcal/mol; this involved various types of interactions, which include conventional hydrogen bonds, Pi-Sigma, Alkyl, and Pi-Alkyl (Figure 7).

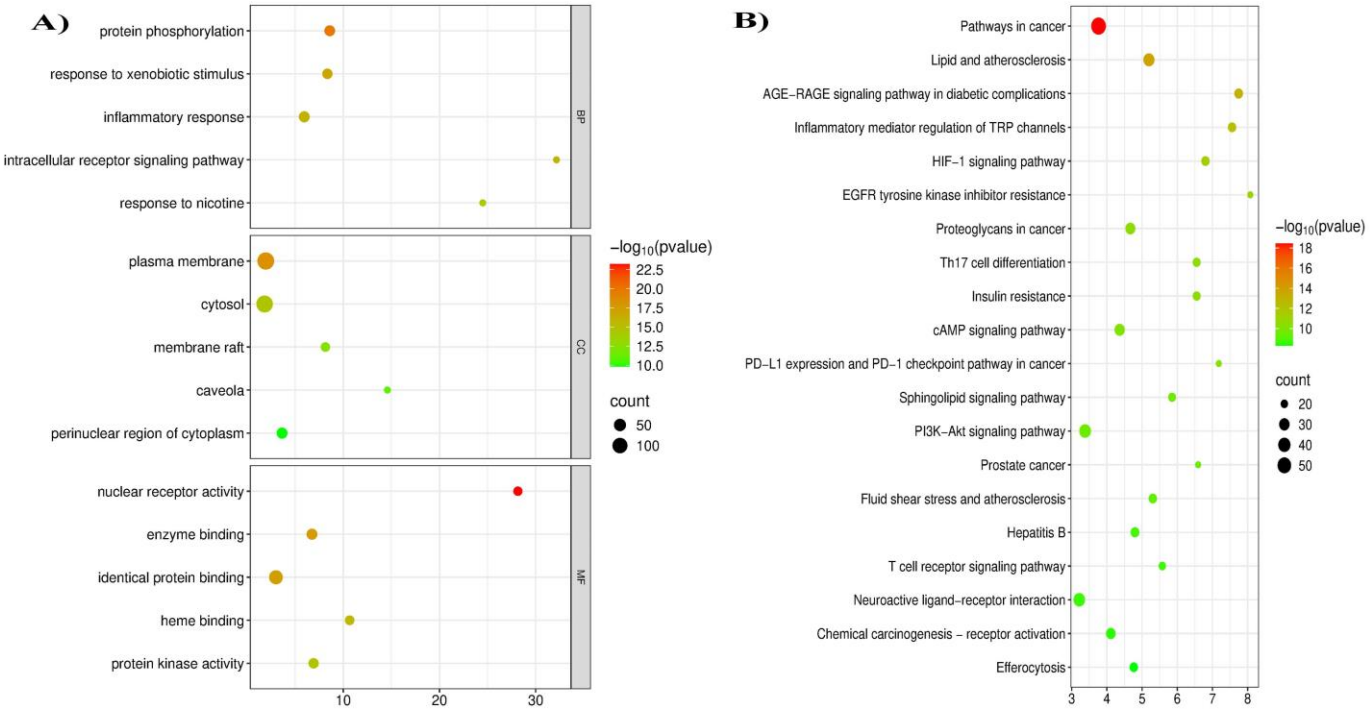

Figure 6. (A) GO enrichment analysis of top pathways; (B) KEGG enrichment analysis of top pathways

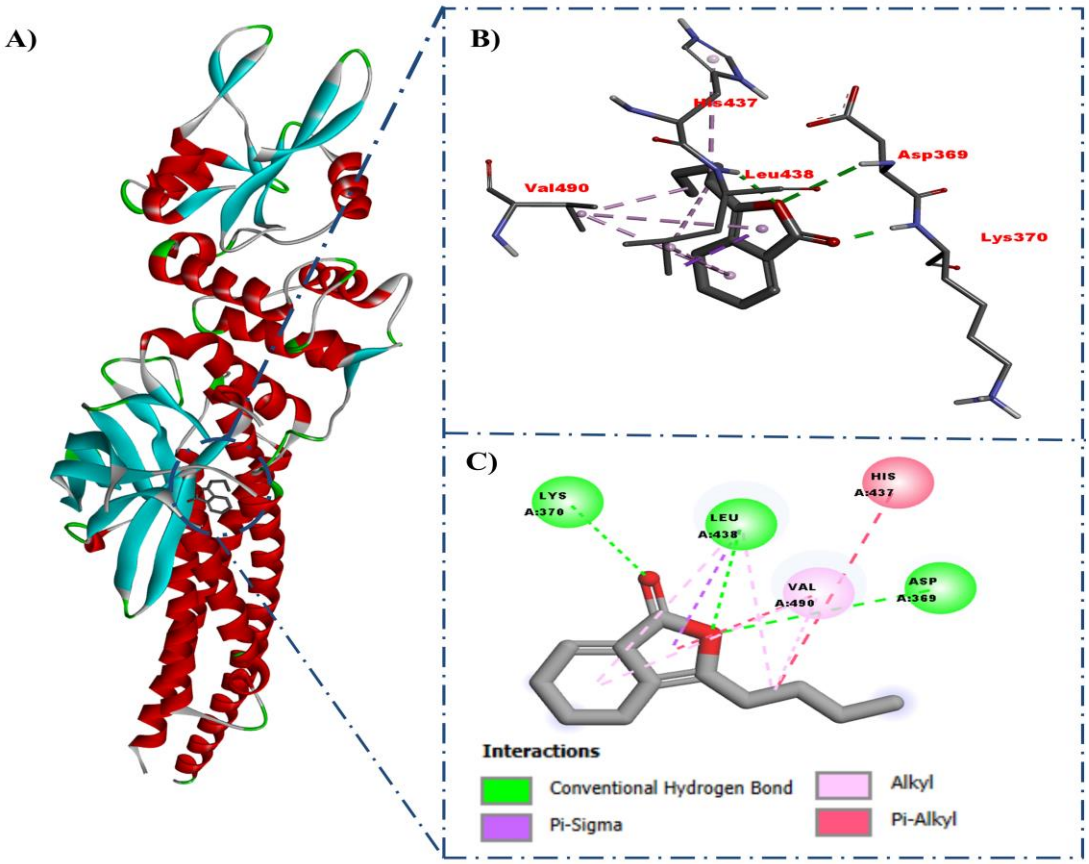

Figure 7. (A) Protein-ligand interactions; (B) Different interactions observed between STAT3 and Z-ligustilide; (C) 2D visualization of interactions between STAT3 and Z-ligustilide

Discussion

The primary objective of this research was to identify the various phytochemicals present in the plant and determine their potential as antioxidants. UV spectral analysis was used as an initial method to determine the presence of these phytochemicals based on their absorption patterns. These preliminary studies were also confirmed by GC-MS studies that defined the secondary metabolites present in the root essential oil of *A. glauca*. Butylidene phthalide, ligustilide, spathulenol, and butylphthalide are the phytochemicals that are reported in an appreciable quantity on GC-MS analysis. Whereas, the UV-Vis spectra of essential oil (Figure 1D) showed peak around 270 nm, a wavelength characteristic for the presence of these compounds, which is consistent with the findings of Lin et al., 1998 study, in which Butylphthalide, E-Ligustilide, and Z-Ligustilide show absorbance around 270 nm (Lin et al., 1998). This correlation between the UV–Vis and GC–MS results strengthen the reliability of the phytochemical analysis and also confirms the abundance of ligustilide in the plant. The SEM-EDX analysis for the root elemental analysis was also investigated, and it was found that there is a large percentage of carbon and oxygen present in the plant material, which indicates the elements present in the portion of the root of the plant. While magnesium, silicon, potassium, and calcium are found in smaller percentages. This work correlates well with the earlier work, in which SEM-EDX elemental analysis shows the presence of oxygen, calcium, copper, magnesium, zinc, sodium, iron, potassium, and selenium in *Ocimum gratissimum* and *O. tenuiflorum* (Sakuntala et al., 2019). The phytochemical profile was evaluated, followed by different *in vitro* assays for phenolics, flavonoid content, and antioxidant activity of *A. glauca* root extracts. It was found from this study that the ethanolic extract possessed the maximum phenolics and flavonoids content than other extractions. This finding is also consistent with UV spectral analysis, which also confirmed the phenolics and flavonoid derivatives. According to Thi et al., (2020) study, the ethanolic extract of *Eryngium foetidum* L. exhibited higher total phenolic content (2.56 ± 0.22 mg GAE/g) and total flavonoid content (55.27 ± 2.77 mg QE/g) compared to the aqueous extract, which is consistent with our findings (Rohilla et al., 2024). Then, the antioxidant capacity of the extracts was evaluated by means of phosphomolybdenum and DPPH free radical scavenging assays. The results showed that both hydroethanolic and ethanolic extracts of the root of *A. glauca* have significantly higher antioxidant activity as compared to all other extracts, which was in agreement with earlier reports. According to Eghdami et al. 2013, *Thymus vulgaris* hydroalcoholic extract possesses a strong antioxidant potential as compared to other solvents. The alcoholic extract showed 25.2±0.75 % inhibition, whereas the hydroalcoholic extract indicated 74.34 ±0.32 % inhibition (Eghdami et al., 2013). According to another study, both ethanol and aqueous extracts exhibited considerable antioxidant activity, with the aqueous extract showing comparatively lower activity (Benbassat et al., 2014). To validate these findings, an *in-silico* analysis was performed. The analysis herein showed that a number of target proteins that including STAT3, HSP90AA1, and EGFR are closely associated with the regulation of oxidative stress. They are involved in several oxidative stress-related pathways essential for cellular homeostasis. In addition, the enrichment analysis of the potential common targets indicated a number of pathways related to the response to oxidative stress such as HIF-1 signaling pathway; AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway and cAMP signaling pathway etc. These paths are associated in regulating the antioxidant response, redox balance, and cell survival under stress conditions. Oxidative stress is caused by an imbalance between the body's antioxidants and free radicals. Superoxide is produced by oxidative enzymes such as cytochrome oxidase and NADH (nicotinamide adenine dinucleotide) or NOX (reduced NADPH oxidase). The AGE-RAGE pathway has been shown to induce oxidative stress mediated by NOX, which in response enhances the redox-sensitive transcription factor NF-B signaling pathway (Sharma et al., 2023). Additionally, the PI3K-Akt signaling pathway stimulates transcription factors such as Nrf2, which result in the upregulation of antioxidant response (Wang et al., 2008; Wu et al., 2022). In another study, it was observed that PI3K, an essential regulatory signaling pathway involved in many physiological processes, phosphorylates its downstream target protein (Akt) to start a signaling cascade. Whereas, Foxo3a regulates ROS that cause apoptosis and participate in oxidative stress. To promote cell survival, Akt phosphorylates Foxo3a, a significant downstream regulator of the PI3K/Akt signaling pathway, resulting in its deactivation (Hossini et al., 2016; Santo et al., 2013; Tan et al., 2022). Thus, the combined results indicate a rich phytochemical and powerful antioxidant nature of *A. glauca* roots. This study primarily focuses on the identification of phytochemicals present in the roots of this plant and their antioxidant potential; this could pave the way for the development of medicines in the future.

Conclusion

The present study results revealed that the roots of *A. glauca* contain various types of secondary metabolites. The *in vitro* and *in silico* approaches revealed its antioxidant potential. Although this study focuses on the antioxidant properties of this plant, its broader medicinal potential remains largely unexplored. Future research should investigate its other pharmacological activities to fully elucidate its therapeutic value. The development of future medicines could benefit from the identification of its bioactive compounds and their mechanisms of action. Therefore, further research into the efficacy, potential, and pharmacokinetic interactions of *A. glauca* could pave the way for future drug development.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable.

AI tool usage declaration

No AI and associated tools are used for writing scientific content in the article.

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